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Synthesis and Biological Evaluation of the [D-MeAla¹¹]-Epimer of Coibamide A

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ABSTRACT

Coibamide A is a highly potent antiproliferative cyclic depsipeptide, which was originally isolated from a Panamanian marine cyanobacterium. In this study, the synthesis of coibamide A has been investigated using Fmoc-based solid-phase peptide synthesis followed by the cleavage of the resulting linear peptide from the resin and its subsequent macrolactonization. The peptide sequence of the linear coibamide A precursor was constructed on a solid-support following the optimization of the coupling conditions, where numerous coupling agents were evaluated. The macrocyclization of the resulting linear peptide provided the [D-MeAla¹¹]-epimer of coibamide A, which exhibited nanomolar cytotoxic activity towards a number of human cancer cell lines.

KEYWORDS

Antiproliferative peptide, coibamide A, depsipeptide, *N*-methyldamino acid

Abbreviations: Hva = α -Hydroxyisovaleric acid; HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; BTC = Bis(trichloromethyl) carbonate; DIC = 1,3-diisopropylcarbodiimide; HOBt = 1-hydroxybenzotriazole; HOAt = 1-hydroxy-7-azabenzotriazole; TFA = trifluoroacetic acid; MSNT = 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole; NMI = *N*-methylimidazole; DCM = dichloromethane.

□ Coibamide A (**1**) is a cyclic depsipeptide, which was originally isolated from a Panamanian

Coibamide A (**1**) is a cyclic depsipeptide, which was originally isolated from a Panamanian marine cyanobacterium (Figure 1).¹ This compound has been reported to exhibit potent antiproliferative activity against a number of human cell lines, where it operates according to a unique mechanism of action. For this reason, there has been considerable interest in the use of coibamide A as a potential lead in cancer drug discovery. The recent results also revealed that coibamide A induces macroautophagy and can trigger apoptosis and non-apoptotic forms of cell death in human cancer cells.² The production and isolation of coibamide A via the cultivation of coibamide A-producing cyanobacterium is a laborious process, and the development of new methods for the chemical synthesis of coibamide A and its derivatives is therefore highly desired to allow for further pharmaceutical research and development.¹ The proposed structure of coibamide A was recently synthesized by He *et al.*,³ but the analytical and biological activity data for the synthetic sample were inconsistent with those reported for natural coibamide A.

Coibamide A has eight *N*-methylamino acids and two ester bonds. The coupling of an amino acid onto the *N*-methylamino terminus of a peptidyl resin proceeds slowly because of steric hindrance, and the synthesis of *N*-methyl-rich peptides can be made difficult by the sluggish nature of this reaction.⁴ The synthesis of *N*-methyl-rich peptides on a solid-support therefore requires the optimization of the reagent used for each coupling step, as exemplified by the syntheses of [MeLeu¹]-cyclosporin A,⁵ the potent nematocide omphalotin A,⁶ the antitumor agent IB-01212⁷ and the immunosuppressive antifungal compound petriellin A.⁸ The coupling conditions developed for the synthesis of [MeLeu¹]-cyclosporin A⁵ involved the use a highly reactive DIC/1-hydroxy-7-azabenzotriazole (HOAt) system⁹ and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU),¹⁰ which led to significant improvements in the yields of the coupling products. Bis(trichloromethyl)carbonate (BTC)

has also been used to improve the coupling efficiency of amidation reactions by converting Fmoc-amino acids into highly reactive and sterically unhindered acid chlorides in situ.⁶

It was envisaged in the current study that coibamide A could be synthesized via the Fmoc-based solid-phase peptide synthesis (SPPS) of a linear peptidyl resin, followed by the cleavage of the resulting linear peptide from the resin and a subsequent macrocyclization reaction between its MeThr⁵ β -hydroxy group and its C-terminal carboxylic acid moiety (Scheme 1). A series of different coupling protocols were investigated in the current study, including the use of DIC/HOAt, HATU and BTC systems to optimize the coupling conditions required for all seven of the *N*-methylamino acid-termini on the solid-support. The progress of each coupling reaction was monitored by HPLC analysis following the cleavage of the peptide product from the peptidyl resin with a 5:95 (v/v) mixture of TFA/DCM.

Tyr(Me)¹⁰ and Ala⁸ were efficiently coupled to *N*-methylamino acids using a HATU/(*i*-Pr)₂NEt system. In contrast, MeLeu⁹ and MeIle⁷ were successfully coupled onto unmethylated amino acids using the standard protocol for Fmoc-based SPPS (i.e., DIC/HOBt). The coupling of MeSer(Me)⁶ onto the *N*-methylamino group of MeIle⁷ in the presence of HATU/(*i*-Pr)₂NEt led to the partial epimerization of the product (dr = 72:28), which was attributed to slow reaction of the two coupling partners under the basic conditions. When the same coupling reaction was conducted with DIC/HOAt, which produces an HOAt ester as the active species without the addition of base, the reaction did not proceed to completion, although no epimerization was observed. In contrast, the coupling of Fmoc-Ser(Me)-OH proceeded smoothly to completion using the same DIC/HOAt reagents. Subsequent deprotection of the Fmoc group followed by an on-resin *N*-methylation protocol using an *o*-nitrobenzenesulfonyl (Ns)-strategy (Scheme 2)¹¹ provided the desired hexapeptide containing MeSer(Me)⁶ in sufficient purity. Overall, this *N*-methylation protocol involved the nosylation of the Ser(Me) α -amino group, *N*-methylation of the resulting nosylated amine using a Mitsunobu reaction and the subsequent deprotection of the Ns-group.

The coupling of Fmoc-Thr(Trt)-OH was also followed by an on-resin *N*-methylation process using the *N*s-strategy described above to give the MeThr(Trt)⁵ residue.

As was the case for MeSer(Me)⁶, the HATU-mediated coupling of MeLeu⁴ did not proceed to completion (61% conversion). The failure of this reaction to reach completion was most likely due to steric hindrance of the *N*-methylamino group and the protected bulky side chain of MeThr(Trt)⁵. Among the various coupling conditions investigated, BTC was found to be the only coupling agent capable of driving the MeLeu⁴ coupling to completion to obtain the desired octapeptide in good purity (83% purity). The second MeSer(Me)³ residue was also incorporated into the linear peptide by the BTC-mediated coupling of Fmoc-MeSer(Me)-OH, which was synthesized according to the Freidinger method.¹² Finally, to allow for the incorporation of the *N*-terminal esterified dimer (Me₂Val¹-Hva²) into the linear peptide resin, the resulting nonapeptide was conjugated to ester **3** using DIC/HOAt; ester **3** was synthesized in advance of this particular step using solution phase chemistry. The resulting peptide was then *N,N*-dimethylated under reductive amination conditions with formalin and NaBH(OAc)₃ to afford the desired linear undecapeptidyl resin **2** in 52% purity.

The final deprotection and cleavage of peptidyl resin **2** was achieved by the treatment of the resin-bound peptide with a 5:95 (v/v) mixture of TFA/DCM, which led to the formation of two products with identical molecular weights (Figure 2). The treatment of the minor product with Et₃N provided only the major product, while the major product underwent partial conversion to the minor product by treatment with TFA/DCM (5:95) (accompanied by the unchanged major product). These suggested that the major product was the desired linear peptide **4**, and that the minor product was the *O*-acyl peptide **5**, which was most likely formed by the rearrangement of the *N*-terminal tetrapeptide from the MeThr⁵ α -amino group to the side-chain hydroxyl group. The desired product **4** could be recovered by the

treatment of the mixture of **4** and **5** with Et₃N followed by purification by reversed-phase HPLC. The overall isolated yield of **4** from resin **1** was 29%.

With the linear precursor **4** in hand, we proceeded to investigate the macrolactonization using LC-MS analysis to monitor the reaction progress. Although various macrolactonization conditions have been reported in the literature,¹³⁻¹⁷ the application of these conditions to peptide **4** failed to provide any of the desired cyclic depsipeptide. Pleasingly, however, the use of MSNT⁷/(*i*-Pr)₂NEt provided access to the desired cyclization product, albeit in a low yield of 3.8%.

The cyclic product was characterized by ESI-MS, MALDI-TOF-MS and ESI-TOF-MS/MS analyses (Figure 3). The expected molecular ions were detected by ESI-MS ([M+2H]²⁺, [M+H]⁺ and [M+Na]⁺) and MALDI-TOF-MS ([M+H]⁺ and [M+Na]⁺). Analysis of the cyclic peptide by ESI-TOF-MS/MS revealed that its fragmentation pattern was identical to that of the natural product, except that an MS/MS fragmentation peak with an *m/z* value of 880 was not observed for the synthetic material. The ¹³C NMR spectrum of the synthetic product was almost identical to that of the natural product (see supplementary data). Although the ¹H NMR spectrum of the synthetic product was similar to that of natural coibamide A (Figure S1), significant differences were observed in the regions spanning δ 1.4–1.7 and 4.5–6.2 ppm (Figure 4). In particular, there were considerable differences between the ¹H signals attributed to MeLeu⁴-β-CH₂, MeSer(Me)³-α-CH and Hva²-α-CH. A comparison of these two compounds by HPLC analysis revealed that the synthetic product eluted slightly ahead of the natural product, which indicated that the synthetic product could be a diastereomer of the natural product. It is entirely possible that the MeAla¹¹ residue may have been epimerized during the macrolactonization step and that only the epimerized precursor underwent the cyclization reaction to provide the corresponding cyclic product, which had an identical molecular weight. The configurations of the hydroxy and amino acids moieties of the synthetic product were therefore investigated using Marfey's analysis (by LC-MS)

and chiral GC-MS using similar methods to those published previously for the analysis of natural coibamide A.¹ Based on the results of this analysis (see supplementary data), the MeAla residue of the synthetic precursor was assigned as D configuration, which confirmed our suspicions that the extended reaction time required for the macrolactonization process had resulted in the racemization and selective cyclization of the linear [D-MeAla¹¹]-undecapeptide.

Initial biological testing of this [D-MeAla¹¹]-epimer of coibamide A revealed that it was potently cytotoxic against A549 (non-small cell lung cancer), HCT116 (colon cancer), MCF-7 (breast cancer) and B16 (murine melanoma) cells at nanomolar concentrations (Table 1). The cytotoxicity of the [D-MeAla¹¹]-epimer of coibamide A was also directly compared with that of natural coibamide A against a panel of four additional human cancer cell lines including H292 (lung carcinoma), MDA-MB-231 (breast cancer), PC-3 (prostate cancer) and SF-295 (glioblastoma) cells. The [D-MeAla¹¹]-epimer of coibamide A showed potent cytotoxic activity towards all four of these cell lines, although the potency exhibited by this material was 3.7- to 8.3-fold less than that of natural coibamide A (Table 2).

In conclusion, we have investigated the synthesis of coibamide A using Fmoc-SPPS followed by the macrolactonization of the resulting linear peptide. The coupling conditions used for the solid-phase synthesis were optimized to obtain the linear precursor in high yield. During the macrocyclization process, only the [D-MeAla¹¹]-epimer was obtained, most likely because of the epimerization of the MeAla¹¹ residue during the slow ester bond formation. The [D-MeAla¹¹]-epimer of coibamide A was found to be slightly less potent than the natural coibamide A, but still exhibited nanomolar cytotoxicity towards a number of different cancer cell lines, and is the first in a series of coibamide A epimers that could be obtained for structure-activity relationship studies.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2014.11.044>.

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Table 1. Cytotoxicities of [D-MeAla¹¹]-coibamide A against human non-small cell lung (A549), colon (HCT116), breast (MCF-7) and murine melanoma (B16) cancer cell lines.^a

cell line	IC ₅₀ (nM)
A549	19.0
HCT116	44.6
MCF-7	48.6
B16	54.4

^aConcentration-response relationships were analyzed using Graphpad Prism Software (Graphpad Software Inc., San Diego, CA), and IC₅₀ values were derived using nonlinear regression analysis fit to a logistic equation.

Table 2. Comparative cytotoxicities for natural coibamide A and the [D-MeAla¹¹]-epimer of coibamide A against human lung carcinoma (H292), breast (MDA-MB-231), prostate (PC-3) and glioblastoma (SF-295) cancer cell lines.^a

cell line	IC ₅₀ (nM)	
	coibamide A (natural product)	[D-MeAla ¹¹]-coibamide A (synthetic product)
H292	124	610
MDA-MB-231	66	545
PC-3	80	424
SF-295	219	816

^aConcentration-response relationships were analyzed using Graphpad Prism Software, and IC₅₀ values were derived using nonlinear regression analysis fit to a logistic equation.

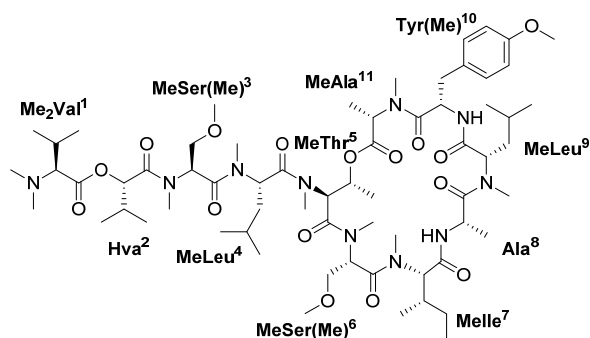


Figure 1. Structure of coibamide A.

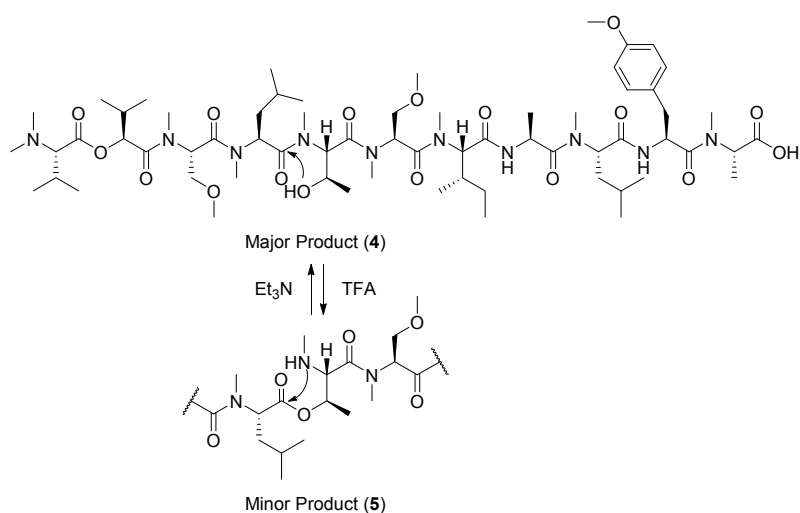


Figure 2. Possible *N*- to *O*-acyl shift at the MeThr⁵ residue following the treatment of linear undeca-peptide **4** with a 5:95 (v/v) mixture of TFA/DCM.

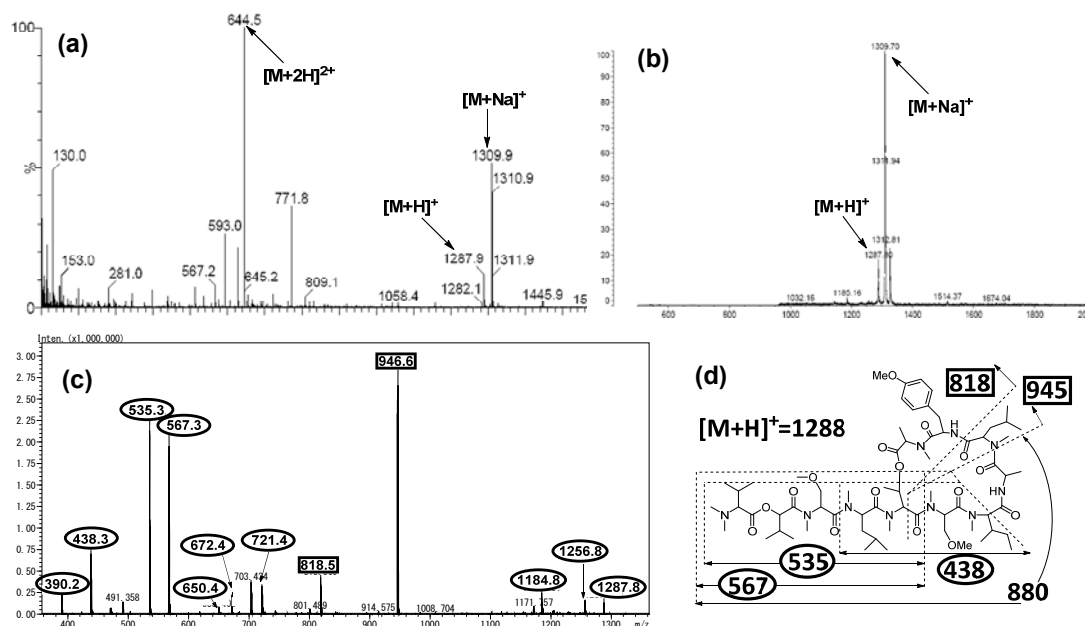


Figure 3. Mass spectrometric analysis of synthetic [D-MeAla¹¹]-coibamide A. (a) ESI-MS spectrum, (b) MALDI-TOF-MS spectrum, (c) ESI-TOF-MS/MS spectrum, and (d) the reported MS/MS fragmentation pattern of natural coibamide A.

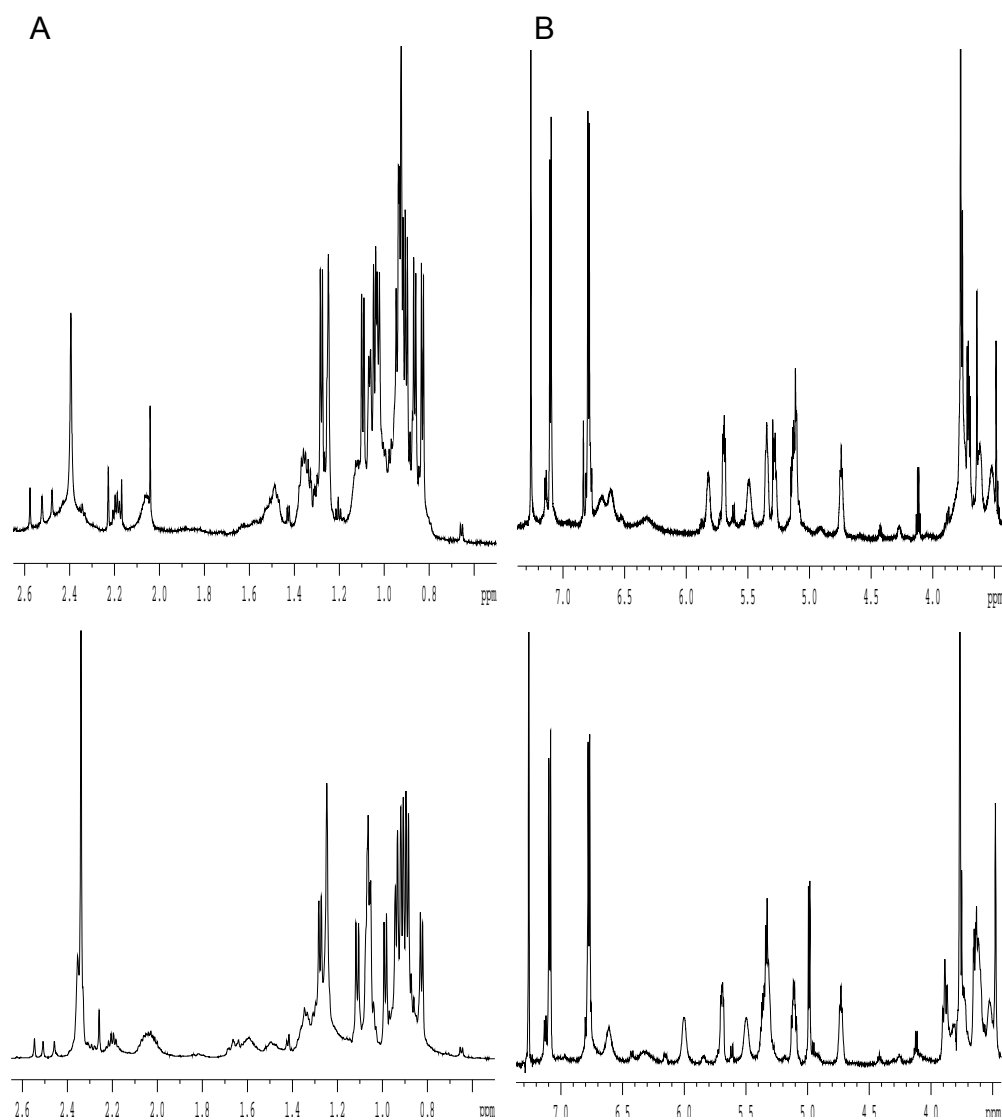
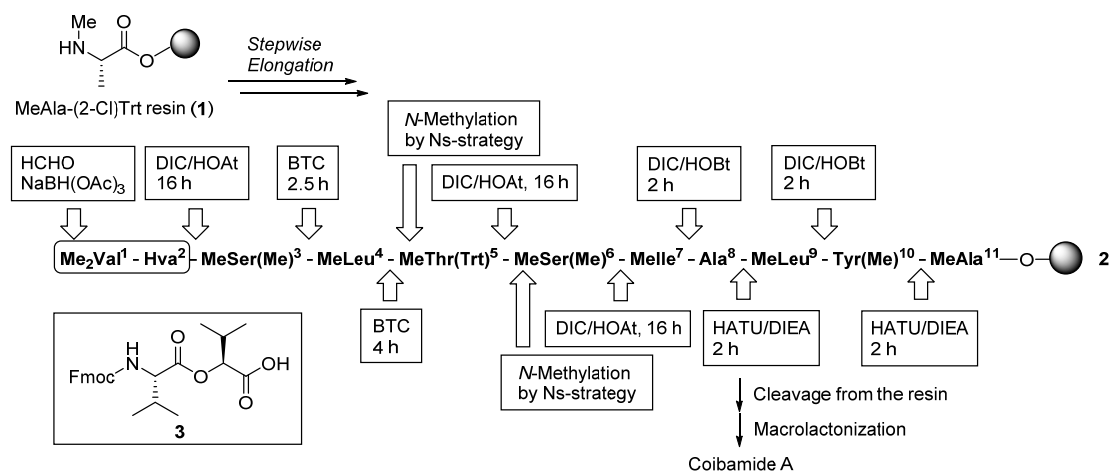


Figure 4. Comparison of A) the alkyl- and upper *N*-methyl and B) the α -proton and aromatic regions of the ^1H NMR spectra for synthetic [D-MeAla^{11}]-coibamide A (upper) and natural coibamide A (lower).

Scheme 1. Overview of the synthetic strategy used for the construction of coibamide A.



Scheme 2. Preparation of *N*-methylamino acids [i.e., MeSer(Me)⁶ and MeThr(Trt)⁵] using the Ns-strategy on a solid support.

